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Corresponding Author: Dr. Stuart Johnson,

Corresponding Author's Institution: Curtin University

First Author: Casiana Villarino

Order of Authors: Casiana Villarino; Vijay Jayasena; Ranil Coorey; Sumana Chakrabarti-Bell; Stuart Johnson

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***Detailed Response to Reviewers**

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Response to reviewer

Please note: Modified text is in blue for this second revision

The authors wish to thank the reviewer for the valuable comments.

Reviewer's comments (line numbers refer to those in the Revised 1 submitted pdf)	**Authors' responses (line numbers refer to those of the Revised 2 copy)**
Reviewer 2	
Lines 212-213 states how the cubes have been cut out The description in lines 212-213 is not sufficient. Crumb of fresh bread is plastic and easily deforms, so it seems impossible to cut ideal cubes out of it, and the inevitable error in such measurements is generally high. This rises a question about cutting method -- was it done with frozen bread, manually or with some device? What about volume measurement (if it was measured e.g. using a laser volume meter, imperfections caused by cutting are not so important)? Please give more details in the text.	L212-216. Further details of how the cube of crumb was cut and measured have been provided as requested.

*Highlights (for review)

Highlights

- Response surface methodology was used to optimise lupin-wheat bread bun quality
- Target was maximum lupin incorporation whilst maintaining consumer acceptability
- Levels of key formulation and process variables identified to give target product
- The “optimal” formulation incorporated lupin at 27g/100 g composite flour

Optimization of formulation and process of Australian sweet lupin (ASL)-wheat bread

Casiana Blanca Jucar Villarino[ac], Vijay Jayasena[a], Ranil Coorey[a], Sumana Chakrabarti-Bell[b] & Stuart Johnson[a,*]

[a] School of Public Health, Faculty of Health Sciences, Curtin University, Bentley, WA 6102, Australia

[b]CSIRO Food Futures National Research Flagship, GPO Box 1600, ACT 2601, Australia

[c] Department of Food Science and Nutrition, College of Home Economics, University of the Philippines, Diliman, Quezon City 1101, Philippines

*Corresponding author. Tel,: +61 8 9266 9486; fax: +61 8 9266 2958

E-mail address: s.johnson@curtin.edu.au (S. Johnson)

Running title: Optimization of ASL-wheat bread

Abstract

This study aimed to optimise formulation and process factors of Australian sweet lupin (ASL)-refined wheat bread bun to maximise the ASL level whilst maintaining bread quality using response surface methodology (RSM) with a central composite face-centered design. Statistical models were generated that predicted the effects of level of ASL flour incorporation (g/ 100 g of ASL-wheat composite flour), ASL-flour volume weighted mean particle size (µm), water incorporation level (g/100 g ASL-wheat composite flour), mixing time of sponge and dough (min) and baking time (min) on crumb specific volume, instrumental texture attributes and consumer acceptability of the breads. Verification experiments were used to validate the accuracy of the predictive models. Optimisation of the formulation and process parameters using models predicted that formulations containing ASL flour at 21.4 - 27.9 g/ 100 g of ASL-wheat composite flour with volume weighted mean particle size of 415 - 687 µm, incorporating water at 59.5 - 71.0 g/100 g ASL-wheat composite flour, with sponges and dough mixed for 4.0 - 5.5 min and bread baked for 10 - 11 min would be within the desirable range of CSV, instrumental hardness and overall consumer acceptability. Verification experiments confirmed that the statistical models accurately predicted the responses.

Keywords: Lupin, wheat, bread, response surface methodology, consumer evaluation

1. Introduction

Australian sweet lupin (*Lupinus angustifolius*, ASL) is a grain legume (pulse) high in protein and dietary fibre. It is a major rotation crop for sustainable farming systems involving wheat and other cereals, due to its nitrogen fixation ability (French, Shea, & Buirchell, 2008). Lupin flour has previously been incorporated into breads (Mubarak, 2001; Doxastakis, Zafiriadis, Irakli, Marlani, & Tananaki, 2002) as well as other baked goods (Nasar-Abbas & Jayasena, 2012). It has been reported that the adding of lupin to refined wheat bread decreased its glycaemic index (Hall, Thomas, & Johnson, 2005) and consumption of lupin-containing foods decreased risk factors for obesity (Lee, Mori, Sipsas, Barden, Puddey, Burke, Hall, & Hodgson, 2006) and cardiovascular disease (Belski, Mori, Puddey, Sipsas, Woodman, Ackland, Beilin, Dove, Carlyon, Jayasena, & Hodgson, 2011) in human clinical studies. However lupin still remains underutilized and undervalued as a food source despite its valuable nutritional and health benefits.

The use of lupin flour in wheat bread results in improved nutritional attributes but can reduce its consumer acceptability as reviewed by Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson (Accepted). This may be a result of the low elasticity of lupin proteins and the high water binding capacity of its dietary fibre (Turnbull, Baxter, & Johnson, 2005) which may weaken the gluten matrix, leading to poor crumb texture and low loaf volume (Guemes-Vera, Pena-Bautista, Jimenez-Martinez, Davila-Ortiz, & Calderon-Dominguez, 2008). Lupin incorporation above 10% results in poor dough and bread quality (Doxastakis, et al., 2002; Mubarak, 2001) but higher levels are desirable to obtain nutritional and health benefits from the lupin-containing bread. There is however a lack of investigations on the effects of formulation and processing parameters and their interaction on lupin-wheat composite flour bread quality and the optimization of the levels of these parameters to maximise the level of lupin incorporation whilst maintaining acceptable bread quality.

Flour particle size and the amount of added water are important formulation parameters that affect bread quality. Previous studies of non-wheat flour substitutes have reported that increased particle size either increased (de Kock, Taylor, & Taylor, 1999) or decreased (Moder, Finney, Bruinsma, Ponte & Bolte, 1984) bread volume. The amount of water added to ASL-wheat bread formulations needs to be carefully adjusted to compensate for the water absorbed by the ASL flour. It has previously been demonstrated that mixing time and baking times were positively associated with bread volume, crumb area and springiness (Villarino, Jayasena, Coorey, Bell, & Johnson, 2014), therefore these factors should also be considered in any optimisation studies.

The mathematical and statistical approach of response surface methodology (RSM) has been used to optimise formulation and process parameters for the manufacture of "healthy" breads such as wholemeal oat bread (Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007), gluten-free breads (McCarthy, Gallagher, Gormley, Schober, & Arendt, 2005) and wheat-legume flour composite breads (Angioloni & Collar, 2012; Jideani & Onwubali, 2009). There is however no published study using RSM to optimise the formulation and process parameters to deliver high quality lupin-wheat composite flour bread with maximum lupin incorporation.

The aim of this study was to use RSM to assess the effects of formulation and process parameters on the physical and sensory qualities of ASL-wheat composite flour bread and to optimize the levels of these parameters to produce acceptable quality bread with maximum level of ASL flour incorporation.

2. Material and methods

2.1. Raw materials

ASL variety *Coromup* was used based on its good performance in previous varietal screening studies of quality of ASL-refined wheat composite flour breads (Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2015). Ten kg of *Coromup* seeds harvested in 2012 at Geraldton, Western Australia were vacuum packed in moisture-proof plastic bags, and stored at ~10^{o}C until use. The seeds were de-coated and milled as previously reported (Villarino, et al., 2014), into flours of three differing target particle sizes (1) 120 µm screen to give 27 µm volume weighted mean particle size; (2) 750 µm screen to give 357 µm volume weighted mean particle size; and (3) 2000 µm screen to give 687 µm volume weighted mean particle size. Screen sizes were determined by preliminary milling experiments. Particle size was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK) as previously reported (Villarino et al., 2014). Flour samples were vacuum-packed in plastic bags and stored in moisture-tight boxes at ~ 10^{o}C until use.

Western Australian refined wheat flour ("baker's flour") was produced by Miller's Food (Byford, WA, Australia). Other bread ingredients i.e. dry yeast (Tandaco, Cerebos Export, Seven Hills, NSW, Australia), bread improver (Healthy Baker, Manildra Group, Gladesville, NSW, Australia), sugar (Coles Brand, Tooronga, VIC, Australia), salt (Coles Brand, Tooronga, VIC, Australia), and vegetable oil (Crisco, NSW, Australia) were purchased from a local supermarket (Coles Supermarket, Perth, WA, Australia).

2.2. Experimental design and statistical analyses

2.2.1. Identifying limits of formulation and processing parameters

The formulation and processing variables evaluated in this study (Table 1) were selected for their potential to influence ASL-wheat bread quality based on findings of previous studies (Flander et al., 2007; Gularte, Gómez, & Rosell, 2012). Their lower and upper limits were chosen as extreme levels at which a bread product could still be manufactured based on preliminary experiments by the authors (data not presented).

2.1.2. Modelling of responses

A central composite face-centered response surface methodology (RSM) design (1/2 fraction) with 5 independent variables and six replicates at the centre point for a total of 32 experimental samples (Table 2) was generated and analysed using Design-Expert Version 8 software (Stat-Ease Inc. Minneapolis, MN, USA). Central composite design is the most common RSM method and is used to estimate coefficients of quadratic models (Stat-Ease Inc., 2011) that can be used for accurate optimisation. The formulation and processing independent variables investigated were: X_1, ASL flour volume weighted particle size (µm); X_2, level of ASL flour incorporation (g/100 g of ASL-wheat composite flour); X_3, level of water incorporation (g/ 100 g composite flour), X_4, mixing time of sponges and dough (min); and X_5, baking time (min). Centre points were replicated to measure reproducibility of the method.

Multiple linear regression analysis was applied to fit data for each response variable to linear and quadratic models. Experimental data were transformed when required based on Box-Cox tests and the most accurate model was chosen through sequential F-tests, lack-of fit tests and other adequacy measures (i.e. R^2, adj R^2, PRESS, DFFITS, DFBETAS, Cook's D). The generalized quadratic equation used for each response variable is given in Eq. 1:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_0 X_i + \sum_{i=1}^{n} \beta_{ii} X_i + \sum \sum_{i<j=1}^{n} \beta_{ij} X_i X_j \qquad \text{(Eq. 1)}$$

where Y is the predicted response; β_0, βi, βii, and βij are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and Xi, and Xj corresponds to the independent variables. Two dimensional contour plots were generated for each response variable, showing the relationship between two independent variables with the three other independent variables fixed at centre levels. Design-Expert Version 8 software (Stat-Ease Inc. Minneapolis, MN, USA) was used for model generation, tests of model adequacy, and contour plot generation. Pearson's Correlation test was used for correlation of bread physical characteristics and were performed using IBM SPSS Statistics V.21 (IBM Corp., NY, USA).

2.2.3. Optimization

Optimization was primarily based on generating a solution with the maximum level of ASL flour incorporation to give maximum CSV, minimum instrumental hardness and minimal consumer overall acceptability of at least 6 ("like slightly"). The secondary optimization objectives were maximum ASL flour particle size and minimum mixing and baking times based on cost minimisation for commercial bread production. Optimization of the formulation and process variables were performed using a multiple response method, "desirability". Desirability is a measure of success when optimising multiple responses and ranges in value from 0 to 1 (least to most desirable, respectively) (Dhinda, Lakshmi, Prakash, & Dasappa, 2012). This approach combined desires and priorities for each of the response and independent variables identified above as the basis of optimization. The desirability scores were generated by the Design-Expert Version 8 software (Stat-Ease Inc. Minneapolis, MN, USA) by specifying the criteria: i.e. goal ("maximise", "minimise", "target", "in range", "equal to"); limits, weights and importance for CSV, instrumental hardness and overall acceptability, ASL flour incorporation, ASL flour particle size, mixing times and baking times (Table 3). Level of ASL flour incorporation was set at maximum as a proxy variable for maximum protein and dietary fibre content of the bread. ASL flour particle size was also specified at maximum level while mixing and baking times were specified at minimum levels. CSV was set at maximum and instrumental hardness at minimum (see Table 3). The target level of overall acceptability by consumer evaluation panel was fixed to a score of 6 ("like slightly") in a 9 point-hedonic scale rating. The limits for CSV and instrumental hardness were based on the upper and lower values determined for wheat-only bread (data not shown). "Weights" for all variables were set at 1. "Importance" for both the ASL flour incorporation and overall acceptability were set at maximum (+++++), since the main objective of the optimization was to maximize ASL incorporation rate whilst maintaining high sensory acceptability of the bread. The software generated the "desirability" scores of different combinations of formulation and process parameters and only scores with >0.70 were considered in the reported optimum range for each variable.

Verification experiments were performed to estimate the predictive capacity of the RSM models. Two bread samples were produced and analysed: one "optimal" and the other "sub-optimal". Experimental data for each response variable were compared to the predicted value of the response using confidence and prediction intervals at α= 0.95. When experimental values of the responses are within the confidence and/or prediction interval the ability of the model to accurately predict responses is validated.

2.3. Bread making

The modified sponge and dough method reported by Villarino et al. (2014) was used for making bread buns. Each baking run comprised of 5 samples namely, a dummy control (wheat bread), internal control (wheat bread), and 3 ASL-wheat bread samples. Formulation and processing conditions at various levels used in the present study are shown in Tables 1 and 2. Doughs were prepared using a total of 550 g of composite ASL- refined wheat flour with water added at various combinations specified in Tables 1 and 2. The amount of water added was based on our previous studies (Villarino et al, 2014; 2105). For each experimental run the wheat sponge contained 30% of the total amount of water while lupin sponge had 55% of the total amount of water and the remaining 15% was added in the dough stage.

Separate sponge preparation for wheat flour and lupin flour was performed. The sponges were proofed for 60 min at 35°C and 80% RH and mixed (using the levels specified in Tables 1 and 2) with other ingredients. The remaining ingredients comprised of 14.3 g yeast, 7.7 g bread improver (Healthy Baker, Manildra Group, Gladesville, NSW, Australia), 5.5 g salt, 5.5 g sugar and 10.4 g vegetable oil and water (15% of the total amount of water). After mixing, the dough was rolled and cut into 50 g bun pieces and proofed for 50 min at 35°C and 80% RH. After proofing the buns were baked at 180°C at specified times in Tables 1 and 2. Physical tests were performed on 3 randomly chosen buns from each treatment after storing at room temperature for up to 24 h after baking. The rest of the buns were frozen at -20 °C and used for evaluation of consumer acceptability. Frozen buns were used in consumer acceptability instead of fresh, due to the logistics of the RSM design. Although freezing might affect the quality of the breads, protocols to minimize the freezing effect (i.e. use of one dedicated freezer, less than a month of frozen storage) and to account for the freezing effect (i.e. presentation of previously frozen wheat-only buns) to each panellist. Other authors have also used frozen bread samples for sensory evaluation of breads (McGuire & O'Palka, 1995).

2.4. Analytical methods

2.4.1. Crumb specific volume (CSV)

Specific volume (cm^3/g) of the crumb was determined in triplicate by carefully cutting a cube from the centre of the bun (after thawing at room temperature overnight in moisture proof packaging), using an electric knife (Kenwood KN400, Delonghi, Australia Pty Limited, Casula Mall, NSW, Australia). The dimensions of the cube were measured using Vernier callipers. Specific volume was calculated as in Eq. 2 as:

$$\text{CSV (cm}^3\text{/g)} = \frac{\text{cube length (cm) x width (cm) x height (cm)}}{\text{cube weight (g)}} \quad \text{(Eq. 2)}$$

2.4.2 Instrumental textural properties

Instrumental textural properties of hardness (g), springiness, cohesiveness and chewiness (g) were measured in triplicate using a TA.XT^{plus} Texture Analyser (Stable Microsystems Ltd., Surrey, UK) with a 5 kg load cell following the methods reported by Villarino et al. (2014).

2.4.3. Consumer evaluation

Two consumer panel groups were used in the study: Group 1 for modelling of the effects of formulation and process parameters and; Group 2 for verification of the models. Group 1 consisted of 74 panellists (14 male and 60 female) and Group 2, 50 panellists (13 male and 37 female). The participants were 18 to 55 years of age, regular bread consumers, not allergic to any food, and not pregnant or lactating. Ethics approval was obtained from the Human Ethics Committee of Curtin University.

During the evaluation of the modelling samples, each panellist (Group 1) received a random selection of nine samples from the total of thirty seven (32 experimental and 5 control samples), served in two sessions, with a 5 min break between each session. Sample presentation was based on a replicated incomplete balanced block design, Plan 13.15 of Cochran & Cox (1957). During the evaluation of the verification samples, each panellist (group 2) evaluated all 3 samples consisting of both crumb and crust of the optimal, non-optimal and control (wheat-only) using a randomized complete block design.

The panellists received 10 g of each sample coded with 3-digit random numbers along and were instructed to evaluate the samples from left to right and to cleanse their palate with water between samples. Panellists rated their acceptability of colour, appearance, flavour/aroma, texture and overall acceptability of the samples using a questionnaire with 9-point hedonic scales (1=dislike extremely; 2=dislike very much; 3=dislike moderately; 4= dislike slightly; 5=neither like nor dislike; 6= like slightly; 7= like moderately; 8= like very much; and 9= like extremely). Evaluations were performed in individual booths illuminated with artificial daylight.

2.5 Proximate and dietary fibre analyses of optimal bread sample

Proximate and dietary fibre analyses were conducted in duplicate or triplicate using standard AOAC Methods (AOAC, 2008) and expressed as g/100 g as is.

3. Results and discussion

3.1. Effects of formulation and process parameters on CSV

The CSV of the ASL-wheat breads ranged from 1.0 to 4.0 cm^3/g. Table 4 shows the effects of formulation and process parameters on CSV expressed as their corresponding regression coefficients in the quadratic models. Tests for reliability of the models (Table 4) indicate that the equations can adequately predict the CSV as a function of the formulation and process factors.

The generated model showed that all formulation and process parameters except for ASL flour particle size had significant ($p<0.05$) effects on CSV. Figure 1(A) presents the contour plot of the effects of level of ASL flour vs level of water incorporation on CSV. This plot illustrates how at a constant level of water incorporation, increasing the level of ASL flour reduces ($p<0.05$) CSV. In addition, at a constant level of ASL flour incorporation, increasing the level of water gives increasing CSV to a maximum, after which further addition of water results in CSV lowering again. This illustrates the quadratic effect ($p<0.05$) of level of water incorporation on CSV.

Published reports have previously demonstrated that above 10% substitution of refined wheat flour by lupin flour decreases bread volume (Dervas, Doxastakis, Hadjisavva-Zinoviadi, & Triantafillakos, 1999; Mubarak, 2001). However, most studies on lupin bread have not considered the effects of other formulation and process parameters and their interaction on bread volume. For instance, in some previous studies, the amount of water used for the lupin-wheat breads and control wheat bread were the same (Guillamon, Cuadrado, Pedrosa, Varela, Cabellos, Muzquiz, & Burbano 2010). However, the quadratic effect of water on CSV observed in the present study and the high water binding capacity of lupin highlight the importance of adding an optimal amount of water to attain desirable ASL-wheat bread volume.

CSV was not significantly associated ($p>0.05$) with either mixing or baking time (Table 4), however the interaction between mixing and baking times (*MT* x *BT*; Table 4) was significant ($p<0.05$), hence the coefficients for the individual factors are included in the model (Table 4) due to the hierarchical conditions of regression models. Figure 1 (B) presents the response surface contour plot of the effect of mixing time vs baking time on CSV. This plot illustrates that mixing time of 4.0-6.4 min with baking time of 10-21 min or mixing time of 5-12 min with baking time of 17.5-25.0 min, give CSV values above the target of 3 cm^3/g. The results indicate that the required gas cell expansion to reach target CSV values of 3 cm^3 /g occurred even at short mixing and baking times.

Given the wide range of possible combinations of mixing and baking times to attain target CSV, it should be possible to minimise these process times to reduce overall bread manufacturing time without comprising the bread quality.

3.2. Effects of formulation and process parameters on instrumental texture

The effects of formulation and process parameters on measures of instrumental texture expressed as their corresponding regression coefficients in the quadratic models are given in Table 4. Tests for reliability of the models (Table 4) generally indicated that the equations can adequately predict the responses as a function of the formulation and process factors. The springiness acceptability model however had a significant ($p<0.05$) lack of fit suggesting it may not be highly accurate. Pearson correlation tests showed significant association between hardness and springiness ($r=-0.79$, $p<0.05$) and hardness and chewiness ($r=0.82$, $p<0.05$). Due to these correlations and that hardness is the most common textural characteristic measured for bread, the following discussion will focus on hardness.

Instrumental hardness of ASL-wheat breads ranged from 256-4834 g and the generated model showed linear, interactive and quadratic associations with formulation and process parameters (Table 4). Figure 2(A) presents the contour plot of the effects of the level of ASL flour vs water incorporation level. This plot demonstrates that there is a limited and specific combination of the amount of ASL flour (~ 16 g /100 g of composite flour) and water ~64 g /100 g of total flour) that is predicted to produce ASL-wheat breads with the target level of hardness (222 g). This limited and specific combination is due to the quadratic effects of both the level of ASL flour and water incorporation and their interaction. The results demonstrate the importance of adding the optimal amount of water to attain desirable ASL-wheat bread texture.

Baking time alone had a quadratic effect on instrumental hardness and particle size of ASL flour had an interactive effect with baking time (Table 4). Figure 2 (B) shows the contour plot of the effects of ASL flour volume weighted mean particle size vs baking time, demonstrating that a minimum ASL flour volume weighted mean particle size of ~192 µm combined with 10 min baking time would produce ASL-wheat breads with the target hardness of < 222 g. The negative linear effect of volume weighted mean particle size on hardness implies that the use of larger ASL flour particle size in ASL-wheat bread results in softer crumb. Larger ASL flour particle size may have resulted in less water absorption (due to their smaller surface area to volume ratio) leading to decreased ability of the ASL flour to compete with the gluten-forming proteins of the wheat flour and improved development of the gluten matrix.

According to de Kock et al (1999) the large flaky shapes of the coarse bran can encapsulate air during the bread making process leading to the more open structure, higher loaf volume and softer and springier crumb. Larger particle size in ASL flour may also have had this type of effect. The interactive effect of ASL flour particle size and baking time might be explained by larger particle size ASL flour giving maximum gas cell expansion during early stages of baking resulting in less time needed for baking to produce softer bread. Likewise, less baking time intuitively would lead to less moisture loss resulting in softer bread.

Based on these findings it appears possible to maximise ASL particle size and minimise baking time to help reduce bread manufacturing costs whilst not compromising the bread quality.

3.3. Effects of formulation and process parameters on consumer acceptability

The effects of formulation and process parameters on consumer acceptability of colour, appearance, flavour, texture and overall acceptability of the breads expressed as their corresponding regression coefficients in the quadratic models are shown in table 5. Tests for reliability (Table 5) indicate that generally the equations can adequately predict these responses as a function of the formulation and process factors. The appearance acceptability model had a significant ($p<0.05$) lack of fit suggesting it may not be highly accurate. Pearson correlation tests show that acceptability of colour, appearance, flavour and texture are all highly correlated ($p<0.05$) with overall acceptability and therefore this discussion will focus on overall acceptability.

Overall acceptability scores of the ASL-wheat breads ranged from 2 (“dislike very much”) to 7 (“like moderately’) and was significantly ($p<0.05$) associated with formulation and process parameters (Table 5). Figure 3(A) shows the contour plot of the effect of level of ASL flour vs water incorporation which indicates that to give the target overall acceptability score of 6, a maximum ASL flour incorporation of ~30 g/100 g composite flour combined with ~68 g water/100 g composite flour is needed. As the level of ASL flour incorporation increases from 5 to 30 g/100 g composite flour there is a corresponding decrease in the range of the amount of water that can be added owing to the quadratic effect of water and its interactive effect with ASL flour incorporation. It can also be observed that the contour plots of the effects of ASL flour vs water incorporation on CSV (Figure 1A) and overall acceptability (Figure 3A) are almost identical. This is reflected in a high Pearson’s correlation ($r=0.88$, $p<0.05$) between CSV and overall acceptability, demonstrating how bread volume is strongly and positively associated with consumer acceptability.

The contour plot of the effect of level of ASL flour incorporation vs mixing time on overall acceptability (Figure 3(B)), demonstrates that a maximum level of ASL flour incorporation of ~28 g/100 g composite flour, mixed for 4 to 12 min, would produce breads with the target minimum overall acceptability score of 6. Decreasing the amount of ASL flour by ~40% (to 17 g/100 g composite flour) combined with a mixing time of 4 to 9.5 would result in an increase in overall acceptability score to 7 (“like moderately”). These results indicate that short mixing times are possible which may assist with the cost-effectiveness of ASL-wheat bread production.

The contour plot of the effect of volume weighted mean particle size of ASL flour vs baking time (Figure 3 (C)) demonstrates that a particle size of > 654 µm combined with a baking time of 10.0 - 23.5 min would produce ASL-wheat breads meeting the target overall acceptability score of 6. Decreasing the particle size below 654 µm reduced the range of baking time that gave breads with overall acceptability score of 6 due to a quadratic effect of baking time and its interactive effect with particle size. The effects of particle size of ASL flour and baking time on overall acceptability may be related to their effects on instrumental illustrated by the high negative correlation ($r=-0.83$, $p<0.05$) between overall acceptability and instrumental hardness. Based on these findings in may be possible to maximise ASL particle size and minimise baking time to reduce costs of ASL-wheat bread manufacturing.

3.4. Optimization and verification of models

The following ranges of optimized formulation and process parameters to meet the optimisation criteria (Table 3) had a "desirability" of >0.70: (a) ASL flour volume weighted mean particle size 415 to 687 µm; (b) level of ASL flour incorporation 21.4 to 27.9 g/100 g composite flour; (c) level of water incorporation 59.5 to 71.0 g/100 g composite flour; (d) mixing time 4.0 to 5.5 min; and (e) baking time 10 to 11 min.

An "optimal" sample was produced with: ASL flour volume weighted particle size 687 µm; ASL flour incorporation 26.8 g/100 g composite flour; water incorporation 66g/100 g composite flour; mixing time 4 min; baking time 10 min. A "non-optimal" sample was produced with: ASL flour volume weighted particle size 122 µm; ASL flour incorporation 26.8 g/100 g composite flour; water incorporation 48 g/100 g composite flour; mixing time of 8 min; baking time 20 min. Photographic images of the "optimal" and "non-optimal" buns are given in Figure 4.

Verification experiments using the "optimal" and "non-optimal" samples demonstrated that that in general, the generated models were able to predict CSV, instrumental hardness and overall acceptability responses (Table 6). Actual values of the sample responses were within the confidence and prediction intervals of the predicted values except for the instrumental hardness of the "optimal" sample.

3.4 Proximate and dietary fibre composition of "optimal" bread sample

The proximate and dietary fibre composition (as is basis) of the "optimal" ASL-wheat bread sample were as follows: protein 19 g/100 g; fat 5 g/100 g; total dietary fibre 19 g/100 g; ash 2 g/100 g; total available carbohydrate 55 g/100 g. The protein and dietary fibre content of the optimal ASL-wheat bread are 62% and 126% respectively higher compared to that of the wheat-only control bread (data not shown), allowing "increased protein" and "good source of dietary fibre" nutrient content claims according to Australia and New Zealand regulations (FSANZ, 2013).

3.5 Conclusion

This study successfully used RSM to model the effects of formulation and process parameters on CSV, instrumental hardness and overall acceptability of ASL-wheat composite flour breads. The statistical models were verified and then used for optimising of the formulation and process parameters to maximise addition of ASL flour in bread for maximum nutritional benefits whilst maintaining acceptable bread quality. Our findings have increased the understanding of the effects of formulation and process parameters on ASL-wheat bread quality. This information will assist the grain industry in providing ASL flour of appropriate specifications for quality bread manufacture to their customers and assist bread manufacturers to develop high quality breads with maximum lupin addition that may assist in consumer nutrition and health. Future research is now required to better understand on one-hand the impact of gluten addition on ASL-wheat bread quality and on the other hand the process and formulation conditions required to manufacture gluten-free ASL based breads to meet this expanding market.

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Table 1. Central composite experimental design showing independent variables with actual and coded values

Factor	Independent variable	Units	Actual values		Coded values	
			Minimum	Maximum	Minimum	Maximum
X1	ASL flour volume weighted mean particle size	µm	27	687	-1	1
X2	Level of ASL flour incorporation	g/100 g composite flour	5	40	-1	1
X3	Level of water incorporation	g/100 g composite flour	40	80	-1	1
X4	Sponge and dough mixing time	min	4	12	-1	1
X5	Baking time	min	10	25	-1	1

ASL, Australian sweet lupin

Table 2. Actual values of formulation and process parameters of the 32 samples used in central composite experimental design

Run	X_1, ASL flour volume weighted mean particle size (µm)	X_2, Level of ASL flour incorporation (g/100 g composite flour)	X_3, Level of water incorporation (g/100 g composite flour)	X_4, Sponge and dough mixing time (min)	X_5, Baking time (min)	Run	X_1, ASL flour volume weighted mean particle size (µm)	X_2, Level of ASL flour incorporation (g/100 g composite flour)	X_3, Level of water incorporation (g/100 g composite flour)	X_4, Sponge and dough mixing time (min)	X_5, Baking time (min)
1	27	40	40	4	10	**17**	687	40	40	12	10
2	27	5	80	4	10	**18**	27	22.5	60	8	17.5
3	687	22.5	60	8	17.5	**19**	27	40	40	12	25
4	357	22.5	40	8	17.5	**20**	687	5	40	12	25
5	687	40	80	12	25	**21**	27	40	80	4	25
6	27	5	80	12	25	**22**	357	22.5	60	8	17.5
7	357	40	60	8	17.5	**23**	687	40	40	4	25
8	357	22.5	60	8	17.5	**24**	357	22.5	60	8	25
9	357	22.5	60	12	17.5	**25**	27	5	40	12	10
10	357	22.5	60	8	17.5	**26**	27	5	40	4	25
11	357	22.5	60	8	17.5	**27**	357	22.5	60	4	17.5
12	687	5	80	4	25	**28**	687	5	40	4	10
13	687	40	40	12	10	**29**	357	22.5	60	8	17.5
14	27	22.5	60	8	17.5	**30**	27	40	80	12	10
15	27	40	40	12	25	**31**	357	5	60	8	17.5
16	687	5	40	12	25	**32**	357	22.5	60	8	10

ASL, Australian sweet lupin

Table 3. Specifications of criteria for the optimization of independent and response variables

Factors	Optimisation criteria			
	Goal	Limits	Weights	Importance
A. Independent variables				
ASL flour incorporation (g/100 g composite flour)	Maximise	5-40	1	+++++
Volume weighted mean particle size µm)	Maximise	27-687	1	+
Mixing time (min)	Minimise	4-12	1	+
Baking time (min)	Minimise	10-25	1	+
B. Dependent variables				
Crumb specific volume (cm^3/g)	Maximise	3.0-5.6	1	+
Instrumental hardness (g)	Minimise	110-222	1	+
Overall acceptability	Target=6	5.5-9.0	1	+++++

Table 4. Effects of formulation and process factors on CSV and instrumental texture of ASL-wheat bread expressed as their corresponding coefficients in the quadratic predictive models

Factor[b]	Crumb specific volume (cm^3/g)	Instrumental texture		
		Hardness (g)[c]	Springiness	Chewiness (g)[c]
Constant	2.267	13.385	0.595	-0.07
PS	-	-0.002*	0.000*	-
LF	0.004*	0.022*	0.006*	0.000*
W	-0.059*	-0.354*	0.002*	0.007*
MT	0.022	0.230	-0.022	-
BT	0.006	0.354*	0.016	-0.011*
PS × *LF*	-	-	-	-
PS × *W*	-	-	-	-
PS × *MT*	-	-	-	-
PS × *BT*	-	0.000*	-	0.000*
LF × *W*	-	-0.000*	-	-
LF × *MT*	-	-	-	-
LF × *BT*	-	-0.002*	-	0.000*
W × *MT*	-	0.055	0.000*	Ns
W × *BT*	-	-	-	Ns
MT × *BT*	-0.001*	-	0.000	
PS^2	-	-	0.000	-
LF^2	-	0.002*	-0.000*	-0.000*
W^2	0.000*	0.003*	-	-0.000*
MT^2	-	-	-	Ns
BT^2	-	-0.008*	-	0.000*
R^2	0.90	0.95*	0.92	0.83
R^2_{adj}	0.88	0.91*	0.88	0.76
CV (%)	7.35	3.72*	3.56	3.41
Lack of fit	0.22	0.10	0.04*	0.22
Transformation	$^{1}/_{\sqrt{Y}}$	*ln(Y)*	*None*	$^{1}/_{\sqrt{Y}}$

*Coefficients significant (95% confidence level)

[b] *PS*, volume weighted mean particle size (µm); *LF*, level of ASL flour incorporation (g/100 g composite flour); *W*, level of water incorporation (g/100 g composite flour); *MT*, mixing time (min); *BT*, baking time; (min)

R^2, R^2_{adj}, *CV (%)* and *Lack of fit are* measures of fit of the model

Transformation is data transformation used to improve fit of models

[c]This is equivalent to 0.0098 N

Table 5. Effects of formulation and process factors on consumer acceptability scores of ASL-wheat bread expressed as their corresponding coefficients in the quadratic predictive models

Factor[b]	Consumer acceptability				
	Colour	**Appearance**	**Flavour**	**Texture**	**Overall**
Constant	1.044	1.051	-5.620	1.045	1.109*
PS	-0.000*	-0.000*	-	-0.000*	0.000
LF	0.004*	0.006*	-0.079*	0.010*	0.008*
W	-0.020*	-0.027*	0.359*	-0.026*	-0.021*
MT	0.006	0.010	-0.115*	0.009	0.007*
BT	0.002*	-0.004*	0.225*	0.006	-0.013
$PS \times LF$	0.000	-	-	0.000*	0.000
$PS \times W$	0.000*	0.000*	-	0.000*	0.000*
$PS \times MT$	0.000*	0.000*	-	-	-
$PS \times BT$	0.000*	0.000*	-	0.000*	0.000*
$LF \times W$	0.000*	0.000*	-	0.000*	0.000*
$LF \times MT$	-0.000*	-0.000*	0.003*	-0.000*	-0.000*
$LF \times BT$	-	0.000*	0.001	-0.000*	-0.000*
$W \times MT$	-0.000*	-0.000*	-	-	-
$W \times BT$	-	-	-	0.000*	-
$MT \times BT$	0.000*	-	-	-	-
PS^2	-	-	-	-	-
LF^2	-	-	-	-	-
W^2	0.000*	-	-0.003*	0.000*	0.000*
MT^2	-	-	-	Ns	-
BT^2	-	0.000*	-0.006*	ns	0.000*
R^2	0.99	0.99	0.90	0.96	0.96*
R^2_{adj}	0.98	0.98	0.87	0.94	0.94*
CV (%)	1.78	4.31	6.61	3.87	3.35*
Lack of fit	0.26	0.02*	0.16	0.21	0.30
Transformation	$1/\sqrt{Y}$	$1/Y$	$(Y)^1$	$1/\sqrt{Y}$	$1/\sqrt{Y}$

*Coefficients significant (95% confidence level)

[b] *PS*, volume weighted mean particle size (µm); *LF*, level of ASL flour incorporation (g/100 g composite flour); *W*, level of water incorporation (g/100 g composite flour); *MT*, mixing time (min); *BT*, baking time; (min)

R^2, R^2_{adj}, *CV (%)* and *Lack of fit are* measures of fit of the model

Transformation is data transformation used to improve fit of models

Table 6. Predicted and actual values of crumb specific volume, instrumental hardness and overall acceptability scores of “optimal” and “non-optimal” ASL-wheat bread.

Response	“Optimal” bread[1]		“Non-optimal” bread[2]	
	Predicted value	Actual value	Predicted value	Actual value
Crumb specific volume (cm^3/g)	3.2±0.0	3.0±0.0	2.0±0.0	2.1±0.0
Hardness (g)	105.1±0.3	198.4±17.5*	1110±0.3	1106.3±145.3
Overall acceptability	6.0±0.0	5.8±2.2	4.6±0.0	5.1±2.2

[1]Conditions: ASL flour volume weighted mean particle size, 687µm; level of ASL flour incorporation, 26.8 g/100 g composite flour; level of water incorporation 66g/100 g composite flour; mixing time of sponge and dough, 4 min; baking time, 10 min

[2]Conditions: ASL flour volume weighted particle size, 122 µm; level of ASL flour incorporation, 26.8 g/100 g composite flour; level of water incorporation, 48 g/100 g composite flour; mixing time of sponge and dough, 8 min; baking time, 20 min

*Denotes significant difference ($p<0.05$) between predicted and actual values for each sample using prediction intervals

Figure legends

Figure 1. Contour plots showing effects on crumb specific volume (cm^3/g) of: (**A**) level of ASL flour and level of water incorporation and (**B**) mixing time and baking time.

Figure 2. Contour plots showing effects on instrumental hardness (g) of: (**A**) level of ASL flour and level of water incorporation and (**B**) volume weighted mean particle size and baking time.

Figure 3. Contour plots showing effects on overall acceptability score of: (**A**) level of ASL flour and level of water incorporation, (**B**) level of ASL flour and mixing time and (**C**) volume weighted mean particle size and baking time.

Figure 4. Photographic images of ASL-wheat bread (optimal and non-optimal) (1) whole bun, and (2) longitudinal cut. (A) level of ASL flour incorporation (g/100 g composite flour), (B) crumb specific volume (cm^3/g), (C) instrumental hardness (g) and (D) overall acceptability score.

Figure
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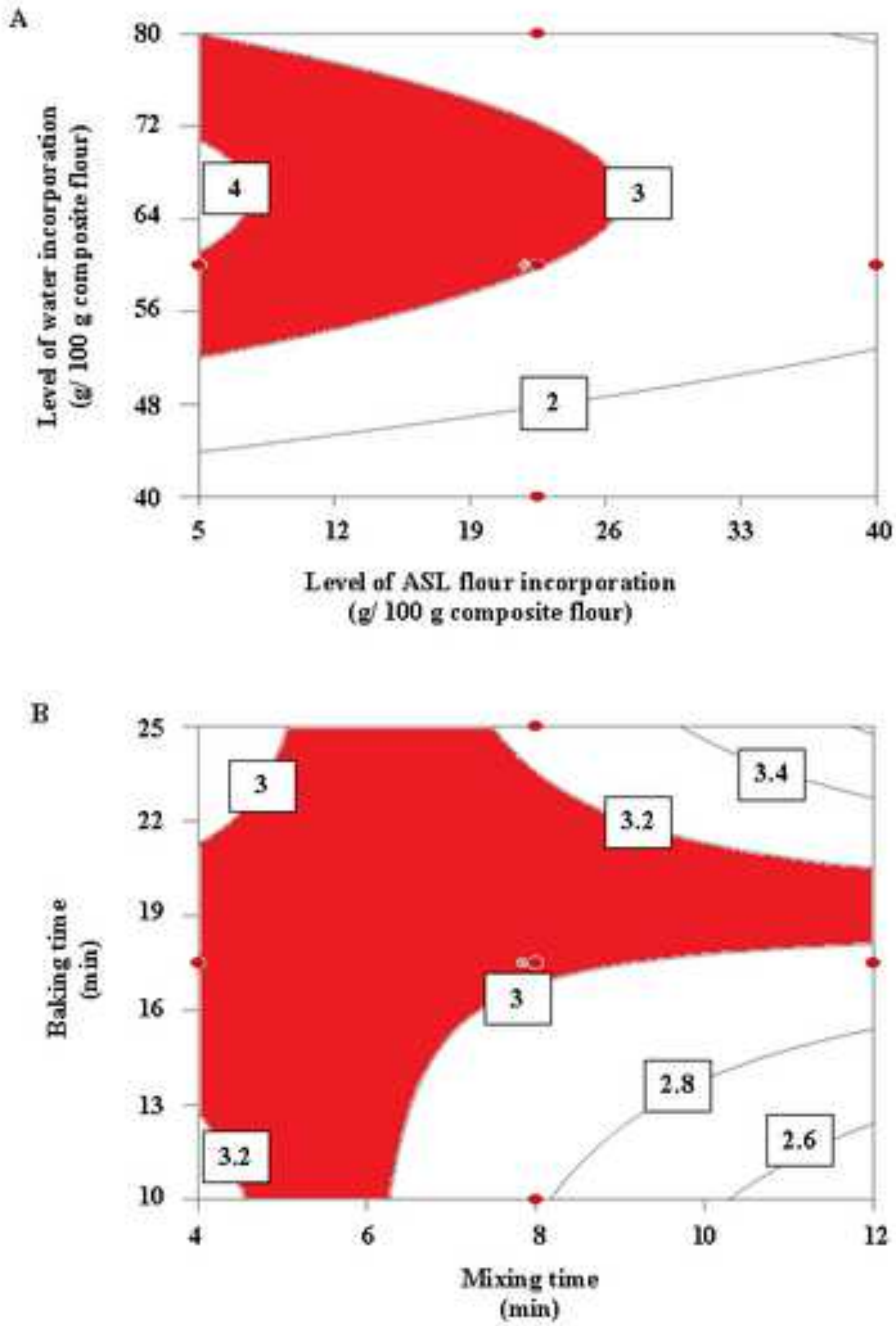

Figure 1

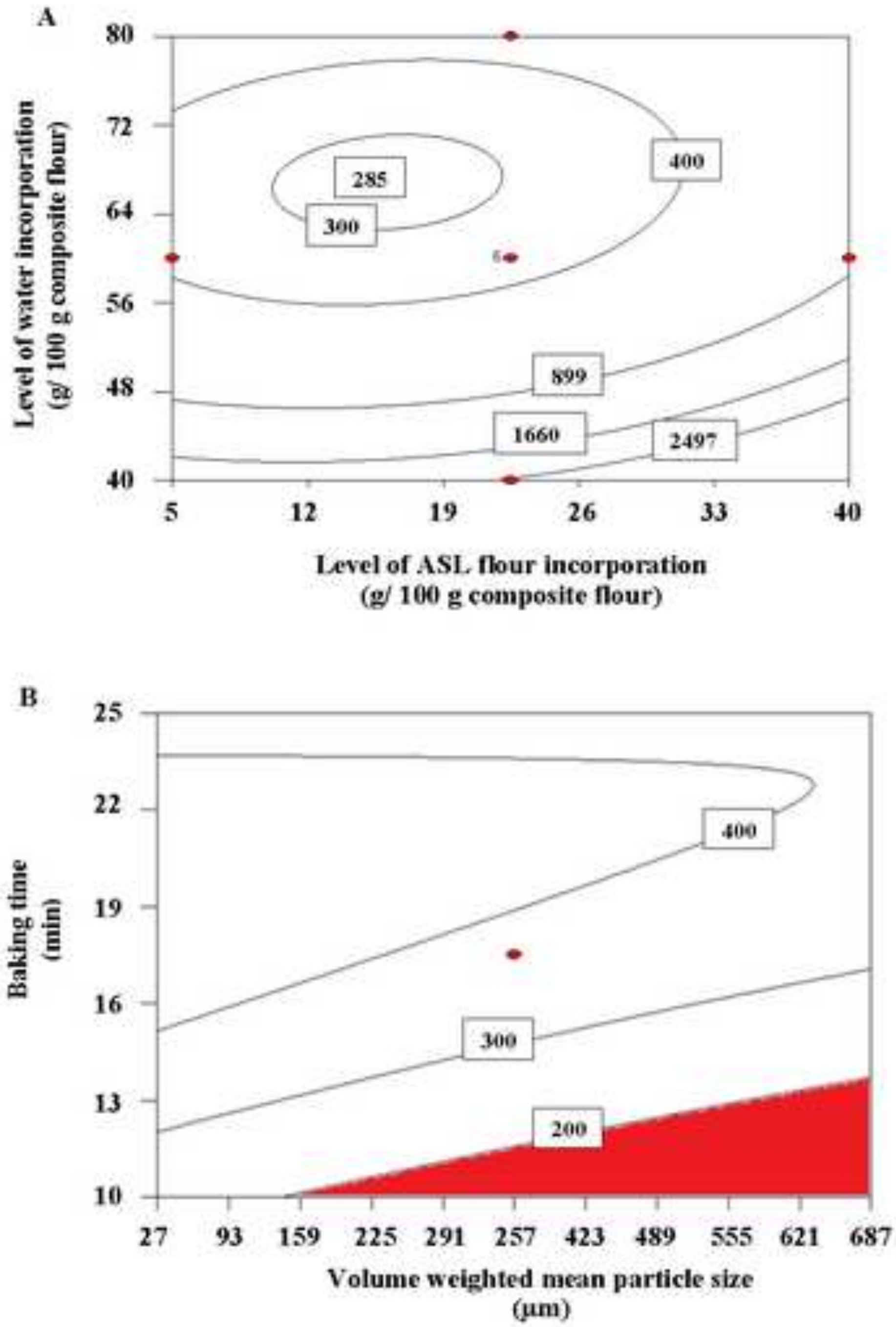
A
80
72
64
56
48
40
5
12
19
26
33
40
Level of water incorporation
(g/ 100 g composite flour)
Level of ASL flour incorporation
(g/ 100 g composite flour)
285
300
400
899
1660
2497
B
25
22
19
16
13
10
27
93
159
225
291
257
423
489
555
621
687
Baking time
(min)
Volume weighted mean particle size
(µm)
400
300
200

Figure 2

Figure 3 p1
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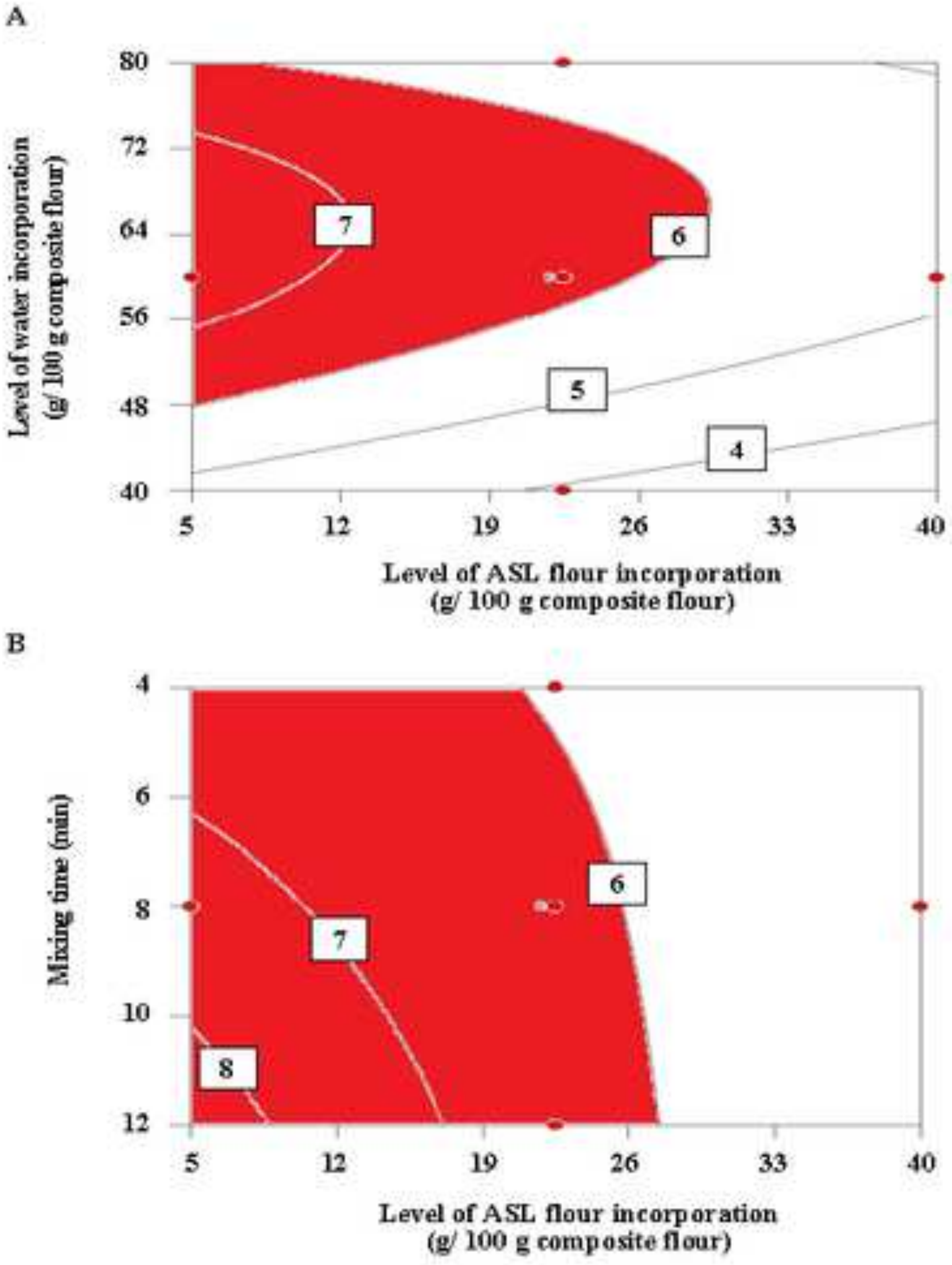

Figure 3 (page 1)

Figure 3 p2
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C

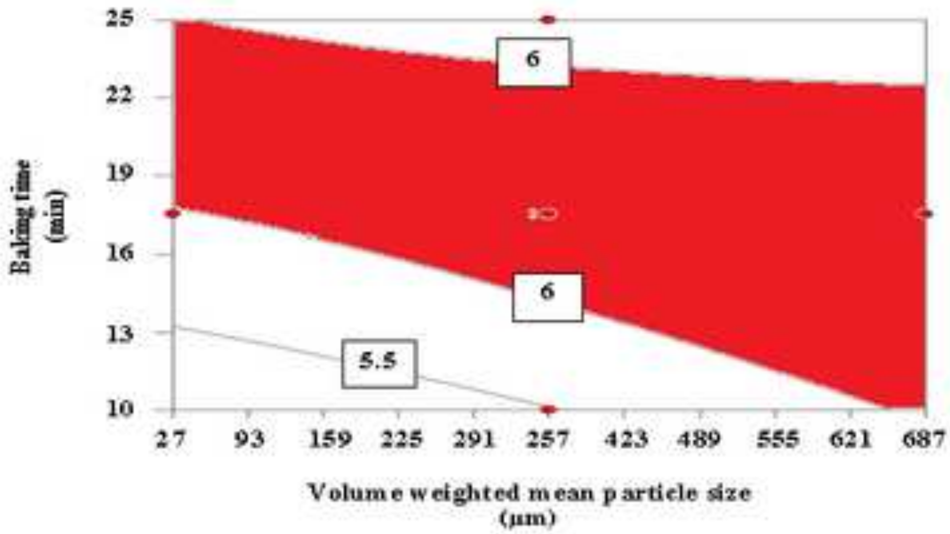

Figure 3 (page 2)

Figure 4
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A	27	27
B	3.0	2.0
C	198	1110
D	6.0	5.0
	OPTIMAL	NON-OPTIMAL

Figure 4